

SPECIATION IN *MIMULUS*, OR, CAN A SIMPLE FLOWER COLOR MUTANT LEAD TO SPECIES DIVERGENCE?¹

Robert K. Vickery, Jr.²

Key words: *Mimulus*, *Erythranthe*, speciation, reproductive isolation, flower color mutations, pollinators, bumblebees, hummingbirds.

The general pattern of speciation in nature has been clear for a long time—the divergence of portions of a population, usually small (Levin 1993), usually in geographic isolation (Mayr 1976), and the accumulation of genetic changes by selection and/or genetic drift (Crow and Kimura 1970) that produce reproductive isolation and normally character divergence as well. The critical step is reproductive isolation, and yet that step—except for polyploid formation which in itself is not always effective (DeWet 1980)—has rarely been observed actually happening in nature. A promising group in which to study speciation events in progress is section *Erythranthe* of the genus *Mimulus* (Vickery 1978).

The six species of monkey flowers comprising section *Erythranthe* are moisture-requiring, herbaceous perennials 1–10 dm in height, with variously shaped, opposite leaves and bilabiate flowers that have four stamens, one style with a bilobed sensitive stigma, and five corolla lobes that range in color from orange to red—rarely yellow—and from lavender-pink to magenta-pink—rarely white. See Grant (1924) for further details. When considered species by species, corollas of *M. cardinalis* Douglas vary from orange to red—rarely yellow—and are sharply and fully reflexed, hummingbird-pollinated flowers. Corollas of *M. verbenaceus* Greene are partially reflexed; that is, the upper two corolla lobes are reflexed, whereas the lower three are gently recurved. Flowers are orange-red to red—rarely yellow—and also are hummingbird-pollinated. Corollas of *M. nelsonii* Grant are partially re-flexed also and have orange-red to red flowers, which are longer than those of *M. verbenaceus* (6–7 cm

versus 4–5 cm). Corollas of *M. eastwoodiae* Rydberg and *M. rupestris* Greene, the two cliff-dwelling species, are partially reflexed, red, and typically hummingbird-pollinated also. And last, flowers of the Rocky Mountain variety of *M. lewisii* Pursh are magenta-pink with all five corolla lobes gently recurved rather than reflexed, thus forming a bee-landing platform; flowers of the Sierra Nevada variety of *M. lewisii* are lavender-pink—rarely white—with corolla lobes thrust forward. Both varieties of *M. lewisii* are bee-pollinated. *Mimulus lewisii* flowers and those of *M. eastwoodiae* and *M. rupestris* produce only modest amounts of nectar, whereas the other species produce abundant nectar (Table 1). Thus, the species differ markedly in flower shape, flower color, nectar production, and, consequently, in pollinators servicing the flowers. In the formation of the six species, evolution appears to have responded to selection imposed by pollinator preferences and ecological opportunities. The result is that members of the complex have radiated into a wide variety of different habitats and niches.

A bright yellow-flowered mutant has appeared on the scene in this setting of pollinator-driven, ecologically opportunistic evolution. In two populations of *M. cardinalis* bright yellow-flowered morphs have become well established. One population is in the Siskiyou Mountains of Oregon, which is the northern limit of the range of *M. cardinalis* (Grant 1924). The other population is on Cedros Island, Baja California, and is at the southern limit of the species range. As Mayr (1976) suggests, new forms often evolve from isolated populations such as these on the periphery of a species

¹The opening talk in the symposium, "Mechanisms of Speciation in Higher Plants," given 1 September 1993 at the XV International Botanical Congress, Yokohama, Japan.

²Biology Department, University of Utah, Salt Lake City, UT 84112 USA.

TABLE 1. Nectar production in the species of section *Erythranthe* measured at 0800 h in the wild (Vickery and Sutherland 1994). Averages are based on 20 or more measurements from a population representative of each species or variety.

Species	Volume in μl	% sugar
<i>M. cardinalis</i>	3.9	11.5
<i>M. verbenaccus</i>	6.7	5.8
<i>M. rupestris</i>	0.9	19.0
<i>M. casticoodiae</i>	1.5	13.7
<i>M. nelsonii</i>	18.3	19.2
<i>M. lewisii</i>		
Rocky Mountains	0.5	0.5
Sierra Nevada	0.7	11.3

range adjacent to new ecological opportunities. A bright yellow-flowered morph of *M. verbenaccus* has appeared also and become well established in a population growing in an isolated spring area, Vasey's Paradise, at the bottom of the Grand Canyon of the Colorado River, AZ, that species' northwestern limit.

Flower colors in section *Erythranthe* are due to various combinations of six anthocyanin pigments—three pelargonidins (apricot-pink) and three cyanidins (lavender-pink)—and at least one carotene pigment (Pollock et al. 1967). The lavender to magenta flowers of *M. lewisii* are due to various combinations of the pelargonidin and cyanidin anthocyanin pigments without the yellow carotene. Flowers of the red-flowered species have all or most of the six anthocyanin pigments plus the carotene pigment. Red color results from a visual blend of pink pigments and yellow pigment. Yellow-flowered plants have a pair of recessive genes at one locus that suppresses anthocyanin production (pink pigments), leaving just the yellow carotene pigment showing. So, a single mutation, when homozygous, changes flower color from red to yellow.

If the change from red to yellow flowers leads to a change in pollinators, for example, from hummingbirds to bumblebees or hawkmoths, then the first major step in reproductive isolation has been established by a single gene change (when homozygous)! Once reproductive isolation has been established by color differences, presumably selection would fine-tune it, e.g., by favoring more tubular flowers for hummingbird-pollinated flowers and by favoring a landing platform morphology and nectar guides for bee-pollinated flowers.

Are pollinators required for seed set in *Mimulus cardinalis* or do the flowers self-pollinate?

To test these two questions, I used the fact that *M. cardinalis* flowers are borne in pairs. I grew plants of red- and of yellow-flowered *M. cardinalis* from Cedros Island in the greenhouse of the Biology Department, University of Utah. The greenhouse is free of pollinators. I carefully hand-pollinated one flower of each of ten pairs of red flowers and of ten pairs of yellow flowers. The hand-pollinated flowers of both the red-flowered and yellow-flowered plants set moderate numbers of seeds per capsule (50–150), while the unpollinated flowers set no seeds at all. This finding corroborates my earlier observations on the Cedros Island *M. cardinalis* (Vickery 1990) that flowers do not self-pollinate and that pollinators are required for seed set.

Are the rewards for pollinators the same in yellow flowers as in red? That is, do yellow flowers and red flowers produce equal volumes of nectar with the same concentrations of sugars? Red flowers of the Cedros Island *M. cardinalis* produced an average (based on flowers from 30 greenhouse-grown plants) volume of 9.5 μl of nectar with 18.2% sugar. Yellow flowers produced an average (based on measurements of flowers from 40 greenhouse-grown plants) of 10.9 μl of nectar with 23.0% sugar. There is so much variation that these values are not significantly different.

Finally, the key question, do pollinators show a preference for red or yellow flowers? To study this question, I placed 24 red-flowered and 24 yellow-flowered plants in a random arrangement in a meadow in the Red Butte Canyon Natural Area in the Wasatch Mountains behind the University of Utah and observed pollinators that visited this experimental population. Pollinators that came were hummingbirds and bumblebees, with rare visits from flies, but no hawkmoths or honey bees. Pollinators were observed for three 50-min periods on each of 5 d. On 28 July 1987 there were 55 hummingbird visits to the 39 red flowers present and 20 visits to the 35 yellow flowers. Chi-square = 14.379, $p < .001$, which indicates a significant preference for red flowers. That day there were 10 bumblebee visits to red flowers and 12 to yellow flowers. Chi-square = 0.1818, no significant preference. On 31 July there were 176 hummingbird visits to the 42 red flowers in bloom that day in the population and 40 visits to the 21 yellow flowers. Chi-square = 70.246, $p < .001$,

which indicates a significant preference for red. That day there were six bumblebee visits to red and one to yellow. There were too few bumblebee visits for a meaningful χ^2 value to be calculated. The same pattern of three observation periods was continued on 2–4 August, but once again there were too few pollinator visits to obtain meaningful χ^2 values. Apparently, most hummingbirds had migrated south and there were few bumblebees all season that year. On the first day of the experiment when the plants had just been placed in the meadow all pollinators would be naive for both red- and yellow-flowered *M. cardinalis* plants inasmuch as Red Butte Canyon is hundreds of miles from the nearest *M. cardinalis* population in northern Arizona. Therefore, the highly significant preference for red appears to be real and not the result of learned behavior. Apparently, hummingbirds strongly preferred the red flowers but also visited the yellow flowers to some extent. The few bumblebee visits did not suggest a preference.

Results show that the change in flower color from red to yellow did affect the frequencies of pollinator visits, but not in an all-or-none way that would immediately establish reproductive isolation. However, the change would probably be enough to initiate partial, incipient reproductive isolation.

Would *M. verbenaceus* with its normal red morph and mutant yellow morph produce the same reactions in pollinators? The flowers of *M. verbenaceus* differ from those of *M. cardinalis* in that only the upper two corolla lobes are reflexed, whereas all five of those of *M. cardinalis* are reflexed. Both species sometimes have wild populations with orange-red flowers instead of the typical red flowers.

For the *M. verbenaceus* experiment, plants of red-flowered and yellow-flowered individuals from Vasey's Paradise in the Grand Canyon plus plants of an orange-red-flowered population from Yecora, Sonora, Mexico, were placed on a lawn by clumps of native Gambel oak at the mouth of Parley's Canyon, Salt Lake City, UT. This location had an abundance of pollinators in contrast to the paucity of pollinators in the Red Butte Canyon meadow used previously. The test population was observed for 15 periods of 1 h each at different times of day from 26 July through 8 August 1988. On average, there were 73 red flowers, 87 orange flowers, and 136 yellow flowers (see Vickery 1990

for daily details of numbers and chi-square calculations). On average, bumblebees visited them 24, 56, and 128 times, respectively; and hummingbirds 43, 98, and 52 times, respectively (Vickery 1990). Bumblebees significantly eschewed red and orange flowers and preferentially visited yellow flowers. Hummingbirds significantly preferred orange, visited red flowers in proportion to their frequency in the population, and significantly eschewed yellow flowers. Results for *M. verbenaceus* are much clearer than those for *M. cardinalis*. There is a definite preference for yellow by bumblebees and a clear avoidance of yellow by hummingbirds. Thus, this color change has led to significant, partial isolation between the normal orange- and red-flowered morphs and the yellow-flowered mutant morph under the conditions of this experiment.

Would *M. cardinalis* react like *M. verbenaceus* in the better experimental locality at the mouth of Parley's Canyon? To probe this question, I added red-, orange-, and yellow-flowered morphs of *M. cardinalis* to the *M. verbenaceus* red-, orange-, and yellow-flowered morphs of the previous experiment. The new experiment was run 8–17 August 1988, with the population being observed for 15 periods of 1 h each at different times of day. On average there were 61 red, 57 orange, and 22 yellow flowers of *M. cardinalis* (see Vickery 1990 for day-to-day numbers and chi-square calculations). On average, bumblebees visited them 28, 30, and 29 times, respectively, and hummingbirds 59, 60, and 6 times, respectively. Bumblebees eschewed red and orange flowers and significantly preferred yellow flowers despite their low numbers in the population. Hummingbirds significantly eschewed yellow flowers and preferentially visited orange flowers. *M. verbenaceus* plants were run again at this time with *M. cardinalis* plants and exhibited the same attractiveness or lack of attractiveness to the pollinators as before. The presence of *M. cardinalis* flowers did not alter pollinator response to *M. verbenaceus* flowers. The color shift from red (or orange) to yellow leads to marked, partial reproductive isolation in *M. verbenaceus* as well as in *M. cardinalis*.

How effective is the partial reproductive isolation? To test this, I placed 198 plants of *M. verbenaceus*—one-sixth yellow-flowered and five-sixths red-flowered to simulate a population with a well-established mutant—in four

experimental areas: the experimental garden on the University of Utah campus, Red Butte Canyon Natural Area, the mouth of Parley's Canyon, and at Silver Fork, Big Cottonwood Canyon, Salt Lake County, UT. I harvested seeds of each plant and planted seeds harvested from 20 yellow-flowered plants and grew them to flowering. If pollinators were visiting the flowers at random, then they should pick up and carry pollen from red flowers five times more often than pollen from yellow flowers. Pollen loads and resulting seed sets were well below the 500–1500 seeds per capsule that may occur in *M. verbenaceus*. So, results were not skewed by saturation of the stigma. Also, assuming all else to be neutral such as relative growth rates of yellow- and red-pollen tubes, speed of flowering of red- and yellow-flowered plants, randomness of placement of red- and yellow-flowered plants, and sample size of red- and yellow-flowered plants, then the expected five-to-one visitation rate should hold. Inasmuch as red is genetically dominant to yellow, then five-sixths of the seedlings should be red-flowered and one-sixth yellow-flowered; that is, of the 214 seedlings grown, 178 should be red-flowered and 36 yellow-flowered. In fact, there were 86 red-flowered seedlings and 128 yellow-flowered seedlings. The ratio is 2 red to 3 yellow flowers, which is far from the expected ratio of 5 red flowers to 1 yellow flower. This suggests considerable pollinator faithfulness to one color or the other. However, in addition to pollinator faithfulness there could be self-pollination. *Mimulus cardinalis* does not self-pollinate but *M. verbenaceus* does at the average rate of 10 seeds per capsule. Average normal seed set is 110 seeds per capsule. Therefore self-pollination would account for 9% of the yellow-flowered seedlings; i.e., 9% of the 214 seedlings, or 19 seedlings, would be expected to be yellow-flowered as a result of self-pollination. Of the remaining 195 seedlings, five-sixths, or 162, would be expected to be red, and one-sixth, or 33, would be expected to be yellow. Therefore, I should expect to observe 162 red-flowered seedlings and 52, i.e., 33 + 19 (the results of self-pollination), yellow-flowered seedlings

instead of the 86 red-flowered and 128 yellow-flowered seedlings actually observed. This is a highly significant difference ($\chi^2 = 146.730$, $p < .0001$) and greatly strengthens the point of pollinator faithfulness. Clearly, pollinator preference for yellow and faithfulness to yellow are having a large effect, though not an all-or-none effect. We are seeing strong incipient reproductive isolation due to color change. In different areas with different conditions and different guilds of pollinators the effect might be less or might be stronger, even leading eventually to effective reproductive isolation and speciation.

ACKNOWLEDGMENTS

I appreciate the financial support of the U.S. National Science Foundation, Grant BSR-8306997. I thank Dr. Stephen Sutherland for nectar measurements and for carrying out the Red Butte Canyon experiment on *M. cardinalis*.

LITERATURE CITED

- CROW, J. E. AND M. KIMURA. 1970. An introduction to population genetics theory. Harper & Row, New York, NY. 591 pp.
- DEWET, J. M. J. 1980. Origins of polyploids. Pages 3–15 in W. H. Lewis, ed., Polyploidy. Plenum Press, Oxford.
- GRANT, A. L. 1924. A monograph of the genus *Mimulus*. Annals of the Missouri Botanical Gardens 11: 99–389.
- LEVIN, D. A. 1993. Local speciation in plants: the rule not the exception. Systematic Botany 18: 197–208.
- MAYR, E. 1976. Evolution and the diversity of life. Harvard University Press, Cambridge, MA. 721 pp.
- POLLOCK, H. C., R. K. VICKERY, JR., AND K. G. WILSON. 1967. Flavonoid pigments in *Mimulus cardinalis* and its related species. I. Anthocyanins. American Journal of Botany 54: 695–701.
- VICKERY, R. K., JR. 1978. Case studies in the evolution of species complexes in *Mimulus*. Evolutionary Biology 11: 404–506.
- . 1990. Pollination experiments in the *Mimulus cardinalis*–*M. lewisii* complex. Great Basin Naturalist 50: 153–159.
- VICKERY, R. K., JR. AND D. SUTHERLAND. 1994. Variance and replenishment of nectar in wild and greenhouse populations of *Mimulus*. Great Basin Naturalist 54: 212–227.

Received 6 July 1994
Accepted 27 September 1994